

What is claimed is:

1. A method for screening a plurality of compounds so as to identify at least one compound exhibiting cognitive enhancing activity, comprising:

- a) determining *in vitro* efficacy and EC_{50} values for each compound at an $\alpha_1\beta_2\gamma_2$ or an $\alpha_5\beta_3\gamma_2$ GABA_A subtype receptor;
- b) determining an *in vitro* efficacy value for each compound at a GABA_A receptor comprising an α_2 or α_3 subunit; and
- c) identifying as exhibiting cognitive enhancing activity a compound having: an EC_{50} value determined in a) of less than about 200nM, an efficacy value determined in a) of less than about -5%, and an efficacy value determined in b) of greater than about 5%.

2. The method of Claim 1 wherein the EC_{50} measured in step a) is less than 150 nM.

3. The method of Claim 2 wherein the *in vitro* efficacy measured at said $\alpha_1\beta_2\gamma_2$ GABA_A subtype receptor or said $\alpha_5\beta_3\gamma_2$ GABA_A subtype receptor is less than -10%.

4. The method of Claim 3 wherein the *in vitro* efficacy measured at said GABA_A receptor comprised of said α_2 subunit or said α_3 subunit is greater than 10%.

5. The method of Claim 1 wherein the *in vitro* efficacy measured at said $\alpha_1\beta_2\gamma_2$ GABA_A subtype receptor or said $\alpha_5\beta_3\gamma_2$ GABA_A subtype receptor is less than -10%.

6. The method of Claim 5 wherein the *in vitro* efficacy measured at said GABA_A receptor comprised of said α_2 or said α_3 subunit is greater than 10%.

7. The method of Claim 1 wherein the GABA_A receptor comprised of said α_2 subunit is an $\alpha_2\beta_3\gamma_2$ GABA_A receptor or the GABA_A receptor comprised of said α_3 subunit is an $\alpha_3\beta_3\gamma_2$ GABA_A receptor.

8. A method for screening compounds for cognitive enhancing activity, comprising:

- a) selecting compounds having a binding affinity less than 100 nM at any GABA_A receptor;
- b) determining *in vitro* efficacy and EC₅₀ values for each selected compound at an $\alpha_1\beta_2\gamma_2$ or $\alpha_5\beta_3\gamma_2$ GABA_A subtype receptor;
- c) determining *in vitro* efficacy and EC₅₀ values for each selected compound at a GABA_A receptor comprised of an α_2 or α_3 subunit; and
- d) identifying as having cognitive enhancing activity any compound having an EC₅₀ value determined in b) of less than 200nM and an efficacy value measured in b) of less than -5%, and an efficacy value measured in c) of greater than 5%.

9. A method of providing a pharmaceutical preparation to patients in need of cognition enhancing treatment comprising:

- 5 a) obtaining at least one compound identified as exhibiting cognition enhancing activity by the method of Claim 1;
- b) testing said at least one compound and submitting results of said testing as part of submission of information under a United States Federal law which regulates the manufacture, use, or sale of drugs or
- 10 veterinary products;
- c) showing a pharmaceutical preparation comprising said at least one compound to be safe for use as required by the provisions of the Federal Food Drug And Cosmetic Act; and
- 15 d) offering the pharmaceutical preparation for sale in the United States of America for use as a cognition enhancing drug or cognition enhancing veterinary product.

20 10. A method for screening a plurality of compounds for cognitive enhancing activity, comprising:

- a) determining *in vitro* efficacy and EC_{50} values for each compound at $\alpha_1\beta_2\gamma_2$ or $\alpha_5\beta_3\gamma_2$ GABA_A receptors;
- 25 b) determining *in vitro* efficacy for each compound at a GABA_A receptor comprised of an α_2 or α_3 subunit;
- c) determining the *in vivo* effect of each compound in an animal model for measuring cognitive enhancement;
- d) determining the *in vivo* effects of each compound in an animal model for proconvulsant activity by measuring
- 30 a seizure threshold in the presence of a seizure inducing compound or in an animal model that predicts anxiogenic effects; and

5 e) identifying a cognitive enhancing compound as a compound having cognitive enhancing properties when the EC₅₀ measured in step a) is less than 200nM and the efficacy measured in step a) is less than -5% and the efficacy measured in step b) is greater than 5% and said compound produces a statistically significant (p <0.05) positive effect in the animal model indicative of cognitive enhancement and said compound does not produce an effect in the animal model predictive of proconvulsant activity of more than a 25% decrease in the seizure threshold in the presence of the seizure inducing drug, or does not produce a change that is statistically significant in said model, or the compound does not produce a statistically significant effect in the animal model that predicts anxiogenic effects.

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20 11. A method for screening compounds for cognitive enhancing properties, comprising:

- 25 a) selecting compounds having binding affinities of less than 100 nM at any GABA_A receptor;
- b) measuring the *in vitro* efficacy of each compound at an $\alpha_1\beta_2\gamma_2$ or $\alpha_5\beta_3\gamma_2$ GABA_A receptor;
- 30 c) measuring the *in vitro* efficacy of each compound at a GABA_A receptor comprised of an α_2 or α_3 subunit;
- d) measuring the *in vivo* effect of each compound in an animal model predictive of cognitive enhancement;
- e) measuring the *in vivo* side effects of each compound in an animal model that predicts proconvulsant activity by measuring a seizure threshold in the presence of a seizure inducing compound or measuring the *in vivo* side

effects of each compound in an animal model that predicts anxiogenic effects; and

f) identifying as a cognitive enhancing compound a particular compound for which the EC_{50} measured in step b) is less than 200nM and the efficacy measured in step b) is less than -5% and the efficacy measured in step c) is greater than 5% and said particular compound produces a statistically significant ($p < 0.05$) positive effect in the animal model indicative of cognitive enhancement and said particular compound does not produce an effect in the animal model predictive of proconvulsant activity of more than a 25% decrease in the seizure threshold in the presence of the seizure inducing drug, or does not produce a change that is statistically significant in said model, or said particular compound does not produce a statistically significant effect in the animal model that predicts anxiogenic effects.

12. A method for screening compounds for hypnotic activity, comprising:

- a) determining EC_{50} and *in vitro* efficacy of each compound at an $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or an $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor;
- b) determining *in vitro* efficacy of each compound at a GABA_A receptor comprised of an α_1 or α_5 subunit; and
- c) selecting a compound having an EC_{50} determined in a) of less than 200nM, an *in vitro* efficacy determined in

a) of greater than 10% for said $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or greater than 50% for said $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor; and an *in vitro* efficacy value determined in b) of less than 50% for the GABA_A receptor comprised of an α_1 subunit or less than 45% for the GABA_A receptor comprised of an α_5 subunit.

13. The method of Claim 12 wherein the *in vitro* efficacy value measured at said $\alpha_2\beta_3\gamma_2$ receptor is greater than 20% or the *in vitro* efficacy value measured said $\alpha_3\beta_3\gamma_2$ GABA_A receptor is greater than 60%.

14. The method of Claim 13 wherein the *in vitro* efficacy value measured at the GABA_A receptor comprised of said α_1 subunit is less than 45% or the *in vitro* efficacy value measured at the GABA_A receptor comprised of said α_5 subunit is less than 40%.

15. The method of Claim 12 wherein the *in vitro* efficacy value measured at the GABA_A receptor comprised of said α_1 subunit is less than 45% or the *in vitro* efficacy value measured at the GABA_A receptor comprised of said α_5 subunit is less than 40%.

16. The method of Claim 12 wherein the EC_{50} measured at said $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or at said $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor is less than 150 nM.

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17. The method of Claim 16 wherein the *in vitro* efficacy measured at said $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor is greater than 20% or the *in vitro* efficacy measured said $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor is greater than 60%.

18. The method of Claim 17 wherein the *in vitro* efficacy measured at the GABA_A receptor comprised of said α_1 subunit is less than 45% or the *in vitro* efficacy measured at the GABA_A receptor comprised of said α_5 subunit is less than 40%.

19. The method of Claim 16 wherein the *in vitro* efficacy measured at the GABA_A receptor comprised of said α_1 subunit is less than 45% or the *in vitro* efficacy measured at the GABA_A receptor comprised of said α_5 subunit is less than 40%.

20. The method of Claim 12 wherein the GABA_A receptor comprised of an α_1 subunit is an $\alpha_1\beta_2\gamma_2$ GABA_A subtype receptor

or the GABA_A receptor comprised of an α_5 subunit is an $\alpha_5\beta_3\gamma_2$ GABA_A subtype receptor.

21. A method for screening a plurality of compounds so as to identify at least one compound exhibiting hypnotic activity, comprising:

- a) selecting a plurality of compounds having a binding affinity of less than 100 nM at any GABA_A receptor.
- b) determining EC₅₀ and in vitro efficacy values for each selected compound at an $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or at an $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor;
- c) determining in vitro efficacy values for each selected compound at a GABA_A receptor comprised of an α_1 or an α_5 subunit; and
- d) identifying as exhibiting hypnotic activity each selected compound having an EC₅₀ value determined in b) of less than 200nM, an in vitro efficacy value measured in b) of greater than 10% for said $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or greater than 50% for said $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor, and an in vitro efficacy value determined in c) of less than 50% for the GABA_A receptor comprised of said α_1 subunit or less than 45% for the GABA_A receptor comprised of said α_5 subunit.

22. A method for screening a plurality of compounds so as to identify compounds exhibiting hypnotic activity, comprising:

- a) measuring the EC_{50} and *in vitro* efficacy of each compound at an $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or an $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor;
- b) measuring the *in vitro* efficacy of each compound at a GABA_A receptor comprised of an α_1 or α_5 subunit; and
- c) measuring the *in vivo* effect of each compound in an animal model indicative of hypnotic effects;
- d) measuring the *in vivo* effect of each compound in an animal model indicative of cognitive impairment; and
- e) identifying a compound as having hypnotic activity when the EC_{50} measured in step a) is less than 200nM, the *in vitro* efficacy measured in step a) is greater than 10% for said $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or greater than 50% for said $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor, and the *in vitro* efficacy measured in step b) is less than 50% for the GABA_A receptor comprised of said α_1 subunit or less than 45% for the GABA_A receptor comprised of said α_5 subunit and said compound produces a statistically significant ($p < 0.05$) positive effect

in the animal model indicative of sedation and said compound does not produce a statistically significant effect in the animal model indicative of cognitive impairment.

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23. A method for screening a plurality of compounds so as to identify at least one compound exhibiting hypnotic activity, comprising:

a) selecting compounds having a binding affinity less than 100 nM at any GABA_A receptor;

b) measuring the EC₅₀ and *in vitro* efficacy of each selected compound at an $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or an $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor;

c) measuring the *in vitro* efficacy of each selected compound at a GABA_A receptor comprised of an α_1 or α_5 subunit; and

d) measuring the *in vivo* effect of each selected compound in an animal model indicative of sedative effects;

e) measuring the *in vivo* effect of each selected compound in an animal model indicative of cognitive impairment; and

f) identifying as having hypnotic activity each selected compound for which the EC₅₀ measured in step

b) is less than 200nM, the *in vitro* efficacy measured in step b) is greater than 10% for said $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or greater than 50% for said $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor, and the *in vitro* efficacy measured in step c) is less than 50% for the GABA_A receptor comprised of said α_1 subunit or less than 45% for the GABA_A receptor comprised of said α_5 subunit and said compound produces a statistically significant ($p < 0.05$) positive effect in the animal model indicative of hypnotic effects and said compound does not produce a statistically significant effect in the animal model indicative of cognitive impairment.

24. A method for screening a plurality of compounds so as to identify compounds exhibiting anxiolytic activity, comprising:

- a) determining *in vitro* efficacy and EC₅₀ value for each compound at an $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or an $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor;
- b) determining *in vitro* efficacy values for each compound at a GABA_A receptor comprised of an α_1 subunit or an α_5 subunit; and

c) identifying as exhibiting anxiolytic activity each compound having an EC_{50} value determined in a) of less than 200nM and an efficacy value measured in a) greater than the efficacy measured in b).

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25. The method of Claim 24 wherein the EC_{50} measured in step a) is less than 150 nM.

26. The method of Claim 25 wherein the *in vitro* efficacy measured at said $\alpha_2\beta_3\gamma_2$ or said $\alpha_3\beta_3\gamma_2$ GABA_A receptor is greater than 20%.

27. The method of Claim 25 wherein the *in vitro* efficacy measured at said $\alpha_2\beta_3\gamma_2$ or said $\alpha_3\beta_3\gamma_2$ GABA_A receptor is greater than 30%.

28. The method of Claim 27 wherein the *in vitro* efficacy measured at said GABA_A receptor comprised of said α_1 or said α_5 subunit is less than 20%.

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29. The method of Claim 24 wherein the *in vitro* efficacy measured at said $\alpha_2\beta_3\gamma_2$ or $\alpha_3\beta_3\gamma_2$ GABA_A receptor is greater than 20%.

30. The method of Claim 24 wherein the *in vitro* efficacy measured at said $\alpha_2\beta_3\gamma_2$ or $\alpha_3\beta_3\gamma_2$ GABA_A receptor is greater than 30%.

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31. The method of Claim 30 wherein the *in vitro* efficacy measured at said GABA_A receptor comprised of said α_1 or said α_5 subunit is less than 20%.

32. The method of Claim 24 wherein the GABA_A receptor comprised of said α_1 subunit is an $\alpha_1\beta_2\gamma_2$ GABA_A subtype receptor or the GABA_A receptor comprised of said α_5 subunit is an $\alpha_5\beta_3\gamma_2$ GABA_A subtype receptor.

33. A method for screening for compounds having anxiolytic activity, comprising:

- a) selecting a compound having a binding affinity less than 100 nM at any GABA_A receptor;
 - b) measuring *in vitro* efficacy and EC₅₀ values for each compound at an $\alpha_2\beta_3\gamma_2$ or $\alpha_3\beta_3\gamma_2$ GABA_A receptor;
 - c) measuring *in vitro* efficacy values for each compound at a GABA_A receptor comprised of an α_1 or α_5 subunit;
- and

d) selecting a compound having an EC_{50} value measured in a) of less than 200nM and an efficacy value measured in b) greater than the efficacy measured in c).

5 34. A method for screening compounds so as to select at least one compound having anxiolytic activity, comprising:

10 a) measuring *in vitro* efficacy for each compound at an $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or an $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor;

15 b) measuring *in vitro* efficacy and EC_{50} values for each compound at a GABA_A receptor comprised of an α_1 or α_5 subunit;

c) measuring *in vivo* effects of each compound in an animal model indicative of anxiolytic activity;

d) measuring *in vivo* effects of each compound in an animal model indicative of sedative effects; and

20 e) selecting each compound having: an EC_{50} value measured in a) of less than 200nM, an efficacy value measured in b) greater than the efficacy measured in step c), a statistically significant ($p < 0.05$) positive effect in the animal model indicative of anxiolytic activity, and no statistically significant effect in the animal indicative of sedative effects.

35. A method for screening a plurality of compounds so as to identify at least one compound having anxiolytic activity, comprising:

- 5 a) selecting a compound having a binding affinity less than 100 nM at any GABA_A receptor;
- b) measuring *in vitro* efficacy and EC₅₀ values for each selected compound at an $\alpha_2\beta_3\gamma_2$ or $\alpha_3\beta_3\gamma_2$ GABA_A receptor;
- c) measuring *in vitro* efficacy for each selected compound at a GABA_A receptor comprised of an α_1 or α_5 subunit;
- d) measuring *in vivo* effects of each selected compound in an animal model indicative of anxiolytic activity;
- e) measuring *in vivo* effect of each selected compound in an animal model indicative of sedative effects; and
- f) selecting a compound having: an EC₅₀ value measured in b) of less than 200nM, an efficacy measured in c) greater than the efficacy measured in d), a statistically significant ($p < 0.05$) positive effect in
- 20 the animal model indicative of anxiolytic activity, and no statistically significant effect in the animal indicative of sedative effects.

36. A method for screening a plurality of compounds so as to identify compounds exhibiting antidepressant activity, comprising:

- 5 a) determining *in vitro* efficacy and EC₅₀ values for each compound using an $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or an $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor;
- b) determining *in vitro* efficacy values for each compound at a GABA_A receptor comprised of an α_1 or an α_5 subunit; and
- 10 c) identifying as having antidepressant activity a compound having an EC₅₀ value determined in a) of less than 200nM and an efficacy value determined in a) of greater than the efficacy value determined in b).

15 37. The method of Claim 36 wherein the EC₅₀ value determined using said $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or said $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor is less than 150 nM.

20 38. The method of Claim 37 wherein the *in vitro* efficacy measured at said $\alpha_2\beta_3\gamma_2$ or said $\alpha_3\beta_3\gamma_2$ GABA_A receptor is greater than 20%.

39. The method of Claim 37 wherein the *in vitro* efficacy measured at said $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or said $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor is greater than 30%.

5 40. The method of Claim 39 wherein the *in vitro* efficacy measured at said GABA_A receptor comprised of said α_1 subunit or said α_5 subunit is less than 20%.

10 41. The method of Claim 36 wherein the *in vitro* efficacy measured at said $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or said $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor is greater than 20%.

15 42. The method of Claim 36 wherein the *in vitro* efficacy measured at said $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or said $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor is greater than 30%.

43. The method of Claim 42 wherein the *in vitro* efficacy measured at said GABA_A receptor comprised of said α_1 subunit or said α_5 subunit is less than 20%.

20 44. The method of Claim 36 wherein the GABA_A receptor comprised of said α_1 subunit is an $\alpha_1\beta_2\gamma_2$ GABA_A subtype

receptor or the GABA_A receptor comprised of said α_5 subunit is an $\alpha_5\beta_3\gamma_2$ GABA_A subtype receptor.

45. A method for screening compounds for antidepressant activity, comprising:

- a) selecting compounds having a binding affinity less than 100 nM at any GABA_A receptor;
- b) determining *in vitro* efficacy and EC₅₀ values for the selected compounds using an $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or an $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor;
- c) determining *in vitro* efficacy for the selected compounds using a GABA_A receptor comprised of an α_1 or an α_5 subunit; and
- d) identifying as having antidepressant activity a compound having an EC₅₀ as determined in b) of less than 200nM and an efficacy value as determined in b) greater than the efficacy value determined in c).

46. A method for screening compounds for antidepressant activity, comprising:

- a) determining *in vitro* efficacy and EC₅₀ values for each compound using an $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor;

b) determining *in vitro* efficacy values for each compound at a GABA_A receptor comprised of an α_1 or an α_5 subunit;

c) determining *in vivo* effect of said compound in an animal model indicative of antidepressant activity;

d) determining the *in vivo* effect of said compound in an animal model indicative of sedative effects; and

e) identifying as an antidepressant a compound that produces an EC₅₀ value as determined in a) of less than 200nM, and an efficacy value as determined in b) greater than the efficacy value from c), and (i) produces a statistically significant ($p < 0.05$) positive effect in the animal model indicative of antidepressant activity and (ii) does not produce a statistically significant effect in the animal model indicative of sedative effects.

47. A method for screening compounds for antidepressant activity, comprising:

a) selecting test compounds having a binding affinity less than 100 nM at any GABA_A receptor;

b) determining *in vitro* efficacy and EC₅₀ value for each test compound using an $\alpha_2\beta_3\gamma_2$ GABA_A subtype receptor or an $\alpha_3\beta_3\gamma_2$ GABA_A subtype receptor;

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c) determining *in vitro* efficacy value for each test compound at a GABA_A receptor comprised of an α_1 subunit or an α_5 subunit;
d) determining the *in vivo* effect of each test compound in an animal model indicative of antidepressant activity;
e) determining the *in vivo* effect of each test compound in an animal model indicative of sedative effects; and
f) identifying as an antidepressant a compound that produces: an EC₅₀ value as determined in b) of less than 200nM, an efficacy value as determined in c) greater than the efficacy value from d), and (i) produces a statistically significant ($p < 0.05$) positive effect in the animal model indicative of antidepressant activity and (ii) does not produce a statistically significant effect in the animal model indicative of sedative effects.

20 48. A method of providing pharmaceutical compounds to patients in need of hypnotic treatment comprising:

- a) obtaining at least one compound identified as exhibiting hypnotic activity by the method of Claim 21;
- b) testing said at least one compound and submitting results of said testing as part of submission of

information under a United States Federal law which regulates the manufacture, use, or sale of drugs or veterinary products

5 c) showing a pharmaceutical preparation comprising said at least one compound to be safe for use as required by the provisions of the Federal Food Drug And Cosmetic Act; and

10 d) offering the pharmaceutical preparation for sale in the United States of America for use as an hypnotic drug or hypnotic veterinary product.

15 49. A method of providing a pharmaceutical preparation to patients in need of anxiolytic treatment comprising:

a) obtaining at least one compound identified as exhibiting anxiolytic activity by the method of Claim 24;

20 b) submitting information regarding the anxiolytic activity of said at least one compound as part of an application under a United States Federal law which regulates the manufacture, use, or sale of drugs or veterinary products

c) showing a pharmaceutical preparation comprising said at least one compound to be safe for use as required by

the provisions of the Federal Food Drug And Cosmetic Act; and

d) offering the pharmaceutical preparation for sale in the United States of America for use as an anxiolytic drug or anxiolytic veterinary product.

50. A method of providing a pharmaceutical preparation to patients in need of antidepressant treatment comprising:

- a) obtaining at least one compound identified as exhibiting antidepressant activity by the method of Claim 36;
- b) testing said at least one compound and submitting results of said testing as part of submission of information under a United States Federal law which regulates the manufacture, use, or sale of drugs or veterinary products
- c) showing a pharmaceutical preparation comprising said at least one compound to be safe for use as required by the provisions of the Federal Food Drug And Cosmetic Act; and
- d) offering the pharmaceutical preparation for sale in the United States of America for use as an antidepressant drug or antidepressant veterinary product.

